MRID No. 427741-09

DATA EVALUATION RECORD

1. **CHEMICAL:** Dicamba.
Shaughnessey No. 029801.

2. **TEST MATERIAL:** Dicamba technical; CAS No. 1918-00-9; Batch No. 52204112; 89.5% active ingredient; a white solid.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Anabaena flos-aquae*.

4. **CITATION:** Hoberg, J.R. 1993. Dicamba Technical - Toxicity to the Freshwater Alga, *Anabaena flos-aquae*. SLI Report No. 93-3-4702. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Sandoz Agro, Inc., Des Plaines, IL. EPA MRID No. 427741-09.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
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Date: 8/12/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
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Signature: P. Kosalwat
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Michael Dary 12/16/94

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant growth and reproduction study. Based on mean measured concentrations of technical dicamba, the 5-day NOEC, LOEC, and EC_{50} for *A. flos-aquae* were 0.005, 0.008, and 0.061 mg ai/l, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

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11. MATERIALS AND METHODS:

A. Test Species: The alga used in the test, *Anabaena flos-aquae*, came from laboratory stock cultures originally obtained from Carolina Biological Supply Company, Burlington, NC. Stock cultures were maintained in sterile Algal Assay Procedure (AAP) medium under test conditions. Transfers were made to fresh medium approximately twice a week. The culture used as the inoculum for the test was transferred to fresh medium three days before test initiation.

B. Test System: Test vessels were sterile 125-ml flasks fitted with stainless steel caps which permitted gas exchange. The vessels were conditioned by rinsing with appropriate test solutions and 50 ml of the test or control solution were placed into each flask. The test medium was the same as that used for culturing with the pH adjusted to 7.4. Test vessels were randomly placed and maintained on an orbital shaker (shaking rate of 100 rpm) under continuous illumination (1.6-2.7 klux at the surface of the medium) in an environmental chamber. The temperature in the chamber was maintained at 24 $\pm$1°C.

C. Dosage: Five-day growth and reproduction test. Based on the results of range-finding tests, seven nominal concentrations of 0.0016, 0.0032, 0.0063, 0.013, 0.025, 0.050, and 0.10 mg active ingredient (ai)/l were selected for the definitive test. The maximum application rate for dicamba was reported to be 4 lb ai/acre, which is equivalent to 2.9 mg ai/l if applied to a 15-cm water column.

A 50 mg ai/l primary stock solution was prepared by dissolving 0.0252 g (as ai) of test material in AAP medium to the final volume of 500 ml. Appropriate volumes of the primary stock solution were diluted to the final volume of 500 ml in AAP medium to prepare the treatment solutions. A medium control was also prepared.

D. Test Design: The test consisted of 3 replicate flasks per treatment level and control. An inoculum of *A. flos-aquae* cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask within 15 minutes of solution addition. The inoculum volume was 0.70 ml per flask. At each 24-hour interval, cell health was assessed and counts were conducted on each

replicate vessel using a hemacytometer and compound microscope.

The conductivity and pH were measured at test initiation and only pH was monitored at test termination. Temperature was recorded continuously with a minimum/maximum thermometer in a flask of water in the environmental chamber. The shaking rate of the orbit shaker and light intensity were recorded daily.

At test initiation and termination, samples were removed from each treatment and control solution for analysis by high performance liquid chromatography. A set of three quality control solutions were prepared at test initiation and termination to monitor the precision and quality control during analysis. Terminal treatment samples were taken from solutions which had been centrifuged (2,000 rpm) for 10 minutes.

E. **Statistics:** The EC_{10}, EC_{50}, and EC_{90} values and their 95% confidence intervals (C.I.) for the 48-, 72-, 96-, and 120-hour test periods were determined by linear regression of response (percent reduction of cell density as compared with the control) vs. mean measured concentration. Various mathematical manipulations (e.g., logarithm and probit transformations) were used on the concentration and response data to obtain the linear regression with the highest coefficient of determination (R^2). The 95% confidence intervals were determined using the method of inverse prediction.

The no-observed-effect concentration (NOEC) was determined to be the highest concentration that caused no significant reduction of cell density in comparison to the control. Williams' test ($p \leq 0.05$) was used to determine significant effects after first checking the data for normality using Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test.

12. **REPORTED RESULTS:** Mean measured concentrations ranged between 120-150% of nominal (Table 3, attached). The mean measured concentrations were 0.0023, 0.0049, 0.0077, 0.014, 0.030, 0.058, and 0.11 mg ai/l. Recoveries of the 0- and 120-hour quality control samples ranged between 90 and 102% of nominal. Analysis of one of the 0-hour quality control samples indicated a recovery of 195%, and this sample value was not used in the determination of the recovery mean.

Cell densities determined at each observation time are presented in Table 4 (attached). At test termination, mean

cell densities in the treatment and control solutions ranged from 60 $x10^4$ to 125 $x10^4$ cells/ml. Cells in the three highest concentration solutions appeared to be bloated and fragmented cells were present in the highest concentration solution. Cell density was significantly reduced at the five highest concentration levels. Cells at the two lowest concentration levels appeared normal. The 120-hour NOEC was determined to be 0.0049 mg ai/l. The 120-hour EC_{50} was determined to be 0.061 mg ai/l (95% C.I.= 0.0096-0.55 mg ai/l).

At test initiation, conductivity ranged from 80 to 100 µmhos/cm. The pH was 7.5 in all treatment and control solutions at test initiation and 7.8-8.0 at test termination.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the study author.

The study director confirmed that this study was conducted in compliance with EPA Good Laboratory Practice (GLP) regulations (40 CFR Part 160) with the exception that maintenance of records on the test substance (characterization and verification) is the responsibility of the sponsor. Additionally, routine water analyses were conducted at an independent laboratory that did not collect data in accordance with GLP procedures. A Quality Assurance statement was included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines with the exception that the level of cellular inoculum (10,000 cells/ml) was greater than recommended (3,000 cells/ml). Additionally, the light intensity (1.6-2.7 klux) was occasionally lower or higher than recommended (2.0 klux).

B. **Statistical Analysis:** Using EPA's Toxanal program and the mean measured concentration data, the reviewer obtained results that were less conservative than those of the author. The reviewer used Williams' test ($p \leq 0.05$) to determine the lowest-observed-effect concentration (LOEC) and NOEC in comparison to the control data. These results were the same as the author's (see attached printouts).

C. **Discussion/Results:** This study is scientifically sound and meets the requirements for a Tier 2 aquatic plant

growth and reproduction study. Based on mean measured concentrations of technical dicamba, the 5-day NOEC, LOEC, and EC_{50} for *A. flos-aquae* were 0.005, 0.008, and 0.061 mg ai/l, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 7-26-93.

Table 3. Concentrations of Dicamba measured in the exposure solutions during the 120-hour toxicity test with *Anabaena flos-aquae*.

Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L)[a]			
	0-Hour	120-Hour	Mean	% Nominal
0.10	0.12	0.11	0.11	120
0.050	0.059	0.056	0.058	120
0.025	0.030	0.030	0.030	120
0.013	0.014	0.015	0.014	110
0.0063	0.0071	0.0082	0.0077	120
0.0032	0.0041	0.0056	0.0049	150
0.0016	0.0020	0.0025	0.0023	140
Control	<0.00098	<0.0014	NA	NA
Stock Solution 50	66	NA	NA	130
QC#1[c] 0.100	0.0903	90.2	0.0920	92.0
QC#2 0.0500	0.0463	92.6	0.0452	90.4
QC#3 0.00200	0.0390[d]	195	0.0020	102

[a] Calculated values are based on actual analytical results and not on rounded values (two significant figures) presented in this table.
[b] NA = Not Applicable
[c] QC = Quality Control sample
[d] The percent recovery for this QC sample was outside of the standard acceptable range established by this laboratory (i.e., within three standard deviations of the mean percent recovery established during the method validation/recovery study, Appendix V). This value was not used in the calculation of the mean percent recovery.

Table 4. **Cell density (x 10^4 cells/mL) of *Anabaena flos-aquae* determined after 24-, 48-, 72-, 96- and 120-hours of exposure to Dicamba Technical.**

Initial Measured Concentration (mg A.I./L)		OBSERVATION INTERVAL (HOURS) 24	48	72	96	120
0.11	A	0	12	19	25	53
	B	8	14	16	27	59
	C	0	20	24	22	69
	Mean(SD)[a]	3(4)	15(4)[b]	19(4)[bc]	25(3)[bc]	60(8)[bcd]
0.058	A	13	12	29	33	55
	B	15	28	27	37	60
	C	6	34	21	38	71
	Mean(SD)[a]	11(5)	24(11)	25(4)[c]	36(3)[c]	62(8)[cd]
0.030	A	11	27	39	45	76
	B	12	38	32	40	53
	C	9	28	34	49	57
	Mean(SD)[a]	10(1)	31(6)	35(3)	44(5)[c]	62(12)[cd]
0.014	A	20	31	49	52	69
	B	14	35	53	58	62
	C	15	39	45	63	88
	Mean(SD)[a]	16(3)	35(4)	49(4)	58(6)	73(13)[d]
0.0077	A	10	22	51	72	97
	B	9	31	54	66	101
	C	11	40	57	63	92
	Mean(SD)[a]	10(1)	31(9)	54(3)	67(5)	97(5)[d]
0.0049	A	16	39	70	76	145
	B	17	25	66	82	109
	C	12	25	65	86	121
	Mean(SD)[a]	15(3)	29(8)	67(3)	81(5)	125(18)
0.0023	A	15	33	76	89	110
	B	11	23	68	90	151
	C	8	38	80	95	107
	Mean(SD)[a]	11(4)	31(8)	75(6)	91(4)	123(24)
Control	A	20	58	79	95	108
	B	29	45	72	82	111
	C	14	38	67	91	142
	Mean(SD)[a]	21(7)	47(10)	73(6)	89(7)	120(19)

[a] Mean and standard deviation (S.D.) are calculated from original raw data, not from the rounded values presented in this table.
[b] Cell fragments were observed.
[c] Bloated cells were observed.
[d] Statistically reduced ($p \leq 0.05$) as compared to the control based on Williams' Test.

anabaena cell density
File: ana Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	120.333	120.333	122.667
2	0.0023	3	122.667	122.667	122.667
3	0.0049	3	125.000	125.000	122.667
4	0.0077	3	96.667	96.667	96.667
5	0.014	3	73.000	73.000	73.000
6	0.030	3	62.000	62.000	62.000
7	0.058	3	62.000	62.000	62.000
8	0.11	3	60.333	60.333	60.333

anabaena cell density
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	122.667				
0.0023	122.667	0.192		1.75	k= 1, v=16
0.0049	122.667	0.192		1.83	k= 2, v=16
0.0077	96.667	1.944	*	1.86	k= 3, v=16
0.014	73.000	3.887	*	1.87	k= 4, v=16
0.030	62.000	4.790	*	1.88	k= 5, v=16
0.058	62.000	4.790	*	1.89	k= 6, v=16
0.11	60.333	4.927	*	1.89	k= 7, v=16

s = 14.914
Note: df used for table values are approximate when v > 20.

NOEL = 0.005 mg ai/l
LOEL = 0.008 mg ai/l

MOSSLER DICAMBA ANABAENA FLOS AQUAE 7-26-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.11	100	50	50	0
.058	100	48	48	0
.03	100	48	48	0
.014	100	39	39	0
.0077	100	19	19	0
.0049	100	0	0	0
.0023	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .11

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	48.71164	.11	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.4699848	8.998408	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.198318

95 PERCENT CONFIDENCE LIMITS = .3768059 AND 2.01983

LC50 = 5.798244E-02

95 PERCENT CONFIDENCE LIMITS = 2.572281E-02 AND .6847423

LC10 = 5.052169E-03

95 PERCENT CONFIDENCE LIMITS = 1.41017E-04 AND 1.251335E-02

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